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ПАТОГЕННОСТ НА ГАЛОВАТА НЕМАТОДА *MELOIDOGYNE HAPLA* ПО КАРТОФИТЕ В БЪЛГАРИЯ
PATHOGENICITY OF THE ROOT-KNOT NEMATODE *MELOIDOGYNE HAPLA* ON POTATO IN BULGARIA

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Резюме

Патогенността и взаимовръзката гостоприемник–паразит на *Meloidogyne hapla* (местна популация от пловдивски картофопроизводителен район) по картофите са проучени при полски и при оранжерийни условия. Картофи от сорта „Адрета" показват типичната реакция на чувствителност към *M. hapla*. По повърхността на нападнатите от *M. hapla* клубени се наблюдават мехурчета, които са ясно диференцирани. По корените се образуват характерните за *M. hapla* гали. Взаимовръзката между началната популационна плътност (Пн) на нематодата и растежа на картофите от сорта „Адрета" е изпитана при оранжерийни условия. Триседмични картофени растения са заразени с 0-64 яйца/cm^3 почва. Осем седмици след инокулацията свежото тегло и общата височина на растението са измерени, определена е крайната нематодна плътност (Пк) на *M. hapla* и степента на галообразуване по корените на растенията. Наблюдава се негативна корелация между Пн и растежа на картофите. Определи се, че прагът на вредност за свежото тегло и височината е съответно 0,5 и 1,0 яйца/cm^3 почва, а при *Пн* = 64 яйца/cm^3 почва минималните относителни стойности - респективно 0,65 и 0,25. Галообразуването по корените на растенията нараства пропорционално с началната популационна плътност на нематодата. Максималният коефициент на размножаване на нематодата – 56,4, е отчетен при Пн = 8 яйца/cm^3 почва.

Abstract

Pathogenicity and host-parasite relationships of *Meloidogyne hapla (local population from the Plovdiv potato growing region)* on potatoes were studied under field and glasshouse conditions. Potato cv. *Adreta* showed a typical susceptible reaction to *M. hapla*. In potato tubers, *M. hapla* induced feeding sites with hypertrophied giant cells. Infection of roots by the nematode resulted in mature galls. The relationship between initial nematode population density and growth of cv. *Adreta* potato seedlings was tested under glasshouse conditions. Three-week-old potato plants were infested with 0 to 64 eggs/cm^3 soil. Eight weeks after inoculation, green mass (shoot weight and height) was measured, nematodes were counted in the potato roots and soil and root galling was assessed. There was a negative correlation between initial nematode densities (Pi) and growing of potato. It is estimated that a threshold limit for fresh shoot weight and height were 0.5 and 1.0 eggs/cm^3 soil, respectively and the minimum possible relative values for fresh shoot weight and height were 0.65 and 0.25, respectively, at *Pi* = 64 eggs/cm^3 of soil. Root galling was proportional to the initial nematode population density. Maximum nematode reproduction rate was 56.4 at a moderate initial population density (Pi = 8 eggs/cm^3 of soil).

Ключови думи: галови нематоди, картофи, *Meloidogyne hapla,* праг на вредност, *Solanum tuberosum.*
Key words: root-knot nematodes, potatoes, *Meloidogyne hapla,* threshold level, *Solanum tuberosum.*

INTRODUCTION

Potato (*Solanum tuberosum* L.) is among the world's most cultivated crops for human consumption. In many areas plant parasitic nematodes, including root-knot nematodes from genus *Meloidogyne*, are among constraints which limit its productivity (Jatala and Bridge, 1990; Brodie et al., 1993; Samaliev and Stoyanov, 2007). From the described more than 80 species root-knot nematodes six have the economical importance on potato: *Ìeloidogyne hapla* Chitwood, *M. incognita* (Kofoid et White) Chitwood, *M.*

javanica (Treub.) Chitwood, *M. arenaria* (Neal) Chitwood, *M. chitwoodi* Golden et al. and *M. fallax* Karssen. *Ì. incognita* is most widely distributed species in countries with subtropical and tropical climate followed by *Ì. javanica* and *Ì. àrenaria,* while *Ì. hapla, Ì. chitwoodi* è *Ì. fallax* are widespread mainly in cooler temperate regions (Brodie et al., 1993; Scurrah et al., 2005). Besides direct yield losses, root-knot nematodes may cause indirect damage in the form of blisters on tubers, as well as brown spots in tuber flesh where the maturing egg mass is located directly under the epidermis.

In recent investigation in Bulgaria, nematode counts in soil and in potato root samples indicated that root-knot nematode, *M. hapla* is widespread in potato fields in major potato producing regions with population density between 20-360 infective larvae per 100 cm^3 soil (Samaliev and Baycheva, 2010; Samaliev, 2011). Microplot studies with the potato cv. Sebago showed that initial densities as low as 670 *M. hapla* larvae per 100 cm^3 soil reduced marketable yield by 49% (Olthof and Potter, 1972). Brodie *et al.*, (1993) reported that in greenhouse condition preplant inoculum thresholds for *M. hapla* in potato were estimated at 50 eggs per 250 cm^3 soil. Information on host-parasite relationships and pathogenicity of root-knot nematodes *Meloidogyne spp.* on potatoes in Bulgaria is lacking.

Therefore, the objective of this study was to conduct a survey to determine the relationship between the initial population density of the *M. hapla* and growth of potato seedlings.

MATERIALS AND METHODS

Identification: Samples of potato tubers together with rhizosphere and bulk soil were taken with a shovel from the upper 25-30 cm of soil from Plovdiv potato growing region (location Sitovo). Second-stage juveniles (J_2) were extracted from roots and soil (Coolen, 1979), and females were recovered from infected root tissues and mounted in glycerin. Glycerin-infiltrated specimens were examined by light microscopy for nematode diagnosis. Morphological criteria used to identify the root-knot population of specimens were: the length and tail tip shape from 50 freshly hatched J_2 per population; perineal patterns (morphology and morphomertric), and distance from base of stylet to branched dorsal esophageal gland orifice (DGO) of 100 mature females. Perineal patterns and anterior body portions were prepared as described by Hartman and Sasser (1985) and examined under a light microscope.

Culture of M. hapla: The root-knot nematode *M. hapla* (location Sitovo) was obtained from cultures derived from single egg masses maintained on tomato (*Lycopersicon esculentum*, cv. *Velosity*) in a greenhouse at 17-25°C. Eggs of *M. hapla* were extracted from roots by using the sodium hypochlorite method (Hussey and Barker, 1973), J_2 were obtained from these eggs by a modified Baermann funnel (Baermann, 1917).

Relationship between inoculum density and plant growth: Top sprouts of the tubers cv. Adreta were removed and planted in a steam sterilised soil (3:1 loam/sand mixture) in a 600 ml plastic pots. The pots were placed in a glasshouse compartment at 17-25°C. Three-week-old potato plants were inoculated with eggs to obtain a range of increasing population densities: 0, 0.25, 0.5, 1, 2, 4, 8, 16, 32 and 64 eggs per cm^3 soil. The treatments were replicated four times and arranged in a randomized complete block design. Plants in pots were watered as needed. During the experiment data on the appearance of symptoms of nematode attack (stunting and yellowing) were recorded. Eight weeks after inoculation, fresh shoot weight and height were measured. After that plants were uprooted and roots were washed free of soil and the roots were stained with Phloxine-B (Dickson and Struble, 1965). The root infection by the nematode was assessed by estimating root gall severity (RGS) on a 0–5 scale: 0 = 0 gall and/or egg masses, 1 = 1-2 galls and/or egg masses, 2 = 3-10 galls and/or egg masses, 3 = 11-30 galls and/or egg masses, 4 = 31-100 galls and/or egg masses and 5 = more than 100 galls and/or egg masses (Taylor and Sasser, 1978). Subsequently, eggs were extracted using the hypochlorite method (Hussey and Barker, 1973) and counted. Nematodes in soil were extracted by a modification of Coolen's method (Coolen, 1979). The final nematode population density (P_f) was calculated as the total number of nematodes extracted from roots and soil.

Statistical analysis: The experiment of relationship between the initial nematode population density and plant growth (indicated by fresh shoot weight and total plant height) was performed twice. Similarity among experiments was tested by preliminary analyses of variance using experimental runs as factors, which allowed the experiment x treatment interaction to be determined. This interaction was not significant ($P_{0.05}$) and allowed data to be combined for analyses of variance. Data on root gall severity (RGS), P_f and reproduction rate (R_f) = final population/initial population, were normalized before analysis by transforming them into log10 (x + 1) (Gomez and Gomez, 1984). Analyses of variance were carried out using SPSS-12 programme. Means of RGS, P_f and R_f values were compared using Fisher's protected least significant-difference test (LSD) at $P_{0.05}$.

RESULTS AND DISCUSSION

Meloidogyne hapla were the only species of root-knot nematode detected in our survey. Patterns were usually delicate with lateral lines, the striae were smooth and parallel, and the arch was flattened to more or less rounded. Punctuations were present around the tail region, whereas wing formation and number varied. Morphological observations based on J_2 (tail length is 55.98±1.65 /55.23-56.16/ μm) and its end is with irregular shape), features of the female perineal pattern (Table 1) and DGO of adult females (5.61±0.03 /5.56-5.70/ μm) matched typical traits of *M. hapla*.

The obtained ìorphotaxonic dates of M. hapla coincide with the data of the populations of this species distributed in our country (Stoyanov, 1980; Samaliev, 1997; Samaliev, 2009; Samaliev and Baycheva, 2010). Knowledge of which species is present in a field, will enable the grower to implement the proper rotation sequence in addition to other control practices, thereby suppressing root-knot nematode populations to increase potato tuber quality.

Potato cv. Adreta plants in commercial fields showed reduced growth, yellowing (Fig. 1a) and heavily damaged roots and tubers. Highly infected tubers were blemished by large masses of parenchymatous tissue (Fig. 1b). Infection of feeder roots by the nematode resulted in mature galls which usually contained at least one mature female and egg mass (Fig. 1c).

Hussey and Williamson (1997) reported that giant cells and tissue modifications induced by nematode infections were found to sequester nutrients from the host plant and limit water and nutrient translocation from infected roots to above-ground plant tissues with subsequent plant growth impairment. The development and parasitic habit of *M. hapla* observed in potato tubers and feeder roots in the present study were similar to those reported for *M. incognita* (Vovlas et al., 1994), suggesting that infection by *M. hapla* has the potential to severely damage potato in Bulgaria.

The reductions of plant growth were negatively correlated with nematode soil population density in the pots. Plant stunting and yellowing caused by *M. hapla,* and reduction of plant shoot growth, were obvious by 4 weeks after inoculation with 16 eggs/cm^3 soil. Eight weeks after inoculation, all plants inoculated with more than 4 eggs/cm^3 soil showed stunting and yellowing. The tolerance limits of potato to *M. hapla* were 0.50 and 1 eggs/cm^3 soil for fresh shoot weight and shoot height, respectively (Fig. 2a and 2b). The minimum possible relative values for fresh shoot weight and height were 0.65 and 0.25, respectively, at Pi = 64 eggs/cm^3 soil (Fig. 2a and 2b).

The statistical processing of the experimental data demonstrated high coefficient of correlation between Pi of *M. hapla* and growing of potato (Table 2). A mathematical model describing the influence of Pi of *M. hapla* on the fresh shoot weight and height was developed on the basis of the obtained results. This influence was described by the following equation:

$$(1) \qquad Y= (a + c\,Pi) / (1 + b\,Pi),$$

where: Y is growth reduction of plant and Pi – initial nematode densities population densities.

The theoretical values of the influence of Pi of *M. hapla* on the fresh shoot weight and height was calculated by this equation. They are statistical indiscernible with the experimentally obtained results (Fig. 2a and 2b, Table 2).

Preplant inoculum thresholds for root-knot nematodes in potato were estimated at 0.004 eggs/cm^3 soil for *M. chitwoodi* (Santo et al., 1981) and 0.2 eggs/cm^3 soil for *M. hapla* (Brodie et al., 1993). Thus, the tolerance limits

Table 1. Measurements (μm) of the perineal patterns of mature females of *M. hapla*

Species	Interphasmidial distance	Vulva width	Anus to center vulva	Anus to tail terminus
Meloidogyne hapla	25.09±0.12 (24.41-26.03)	18.90±0.07 (17.56-21.12)	23.19±0.11 (22.59-23.70)	9.60±0.07 (8.18-10.05)

*n = 100

(a) (b) (c)

Fig. 1. *A heavily infected patch within a potato cv. Adreta by Meloidogyne hapla. (a) Plants are stunted, and yellow more rapidly than those in more lightly infested parts of the field; (b) Potato tubers blemished by parenchymatous tissue growth caused by high densities of Meloidogyne hapla; (c) Potato roots with galls and egg masses of M. hapla*

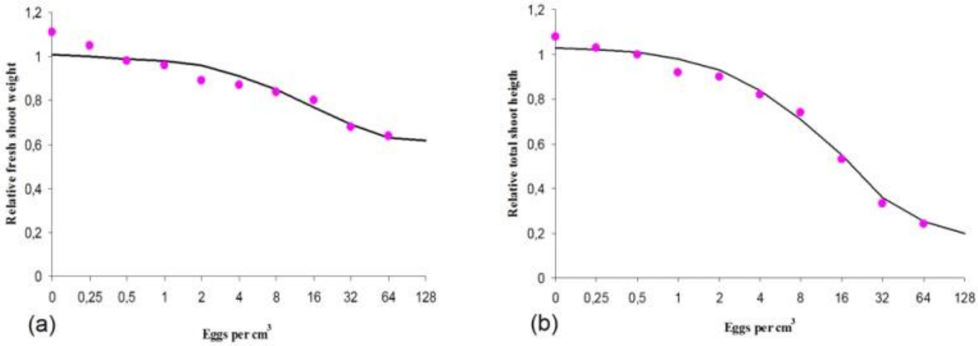

Fig. 2. *Relationship between initial population densities (Pi) of Meloidogyne hapla and relative fresh shoot weight (a) and shoot height (b) of potato cv. Adreta plants grown in pots at 17-25°C in the glasshouse for eight weeks*

Table 2. An estimate of the parameters with two models describing the relationship between fresh shoot weight/height and the initial population density of *Meloidogyne hapla*

Variants (equation)	Coeficients			Stat. characteristics	
	A	b	c	r^2	Fit Std. Err.
Fresh shoot weight	1.01	0.07	0.03	0.90	0.05
Fresh shoot height	1.03	0.05	0.001	0.97	0.04

Table 3. Relationship between initial population density of *Meloidogyne hapla* and root galling, final population density and reproduction rate on seed potato cv. Adreta, eight weeks after inoculation

Initial population density (Pi)/g soil	RGS**	P_f	R_f***
0.25	1.6 c	688 e*	4.6 d
0.5	2.8 b	4521 d	15.0 c
1	3.1 b	7396 d	18.3 c
4	4.2 a	128542 b	53.6 a
8	4.4 a	270600 b	56.4 a
16	4.7 a	495124 a	51.6 a
32	4.8 a	529670 a	27.7 b
64	5.0 a	421112 a	11.0 c

*Means followed by the same letter do not differ significantly ($P_{0.05}$) according to Fisher's protected LSD test; **Severity of root galling (RGS) was rated on a 0–5 scale: 0, no galls; and 5, completely deformed root system with a few large galls; ***R_f (nematode reproduction rate) = P_f (final nematode population per plant)/Pi

in the present study of 0.5 and 1 egg/cm³ soil for fresh shoot weight and shoot height of potato cv. Adreta, respectively, indicate that under conditions conducive to infection local population of *M. hapla* from Plovdiv potato growing region is less aggressive on potato than *M. chitwoodi* and *M. hapla with* damage on potato that has been reported in the northeastern and north-central USA, eastern and central Canada, and in the Netherlands. This is probably a consequence of the highly variable genetic make-up of *Meloidogyne species.*

The maximum nematode reproduction rate (R_f) was 56.4 at a moderate initial population density of 8 eggs/cm³ soil. In general, the reproduction rate increased as the initial nematode population increased up to 1 egg/cm³ soil, and decreased at the highest initial nematode population (Table 3).

The reduction of nematode reproduction rate with increasing initial nematode inoculum density has been reported for infections of several crops by *Meloidogyne* spp. (Di Vito et al., 1986, 2004). Reproduction rates of local population of *M. hapla* on potato at an initial inoculum density of 1 egg per cm^3 soil were similar to those observed for *M. chitwoodi,* and higher than those for *M. fallax* or *M. hapla,* on several potato cultivars (Van der Beek et al., 1997).

CONCLUSIONS

There was a negative correlation between initial nematode densities (Pi) and growing of potato. Thresholds limit for fresh shoot weight and shoot height were 0.5 and 1.0 eggs/cm^3 soil, respectively. Minimum possible relative values for fresh shoot weight and height were 0.65 and 0.25, respectively, at Pi = 64 eggs/cm^3 soil. Root galling was proportional to the initial nematode population density. Maximum nematode reproduction rate was 56.4 at a moderate initial population density (P = 8 eggs/cm^3 soil).

In view of the aggressiveness of *M. hapla* to potato demonstrated in the present study, management of this nematode species should be considered when implementing integrated pest management programs in potato-growing areas where environmental conditions and potato-culture practices may favour the development of *M. hapla.* The results also highlight the need for additional studies on the resistance of potato to *M. hapla.*

REFERENCES

Baermann, G., 1917. Eine einfache Methode zur Auffindung von Ancylostomum (Nematoden) Larven in Erdproben. Gennesk. – Tijdschr. Ned. Ind., 57:131-137.

Brodie, B.B., K. Evans, J. Franco, 1993. Nematode parasites of potatoes. – In: Evans K, Trudgill D.L., J.M. Webster, eds. Plant Parasitic Nematodes in Temperate Agriculture. Wallingford, UK: CAB International, 87–132.

Coolen, W.A., 1979. Methods for the extraction of *Meloidogyne* spp. and other nematodes from roots and soil. – In: Lamberti F, Taylor CE, eds. Root-Knot Nematodes *(Meloidogyne* Species) Systematics, Biology and Control. London, UK: Academic Press, 317–329.

Di Vito, M., N. Greco, A. Carella, 1986. Effect of *Meloidogyne incognita* and importance of the inoculum on the yield of eggplant. – Journal of Nematology, 18, 487–490.

Di Vito, M., N. Vovlas, P. Castillo, 2004. Host-parasite relationships of *Meloidogyne incognita* on spinach. – Plant Pathology, 53, 508–514.

Dickson, D.W., F.B. Struble, 1965. A sieving staining technique for the extraction of egg masses of *Meloidogyne incognita* from soil. – Phytopathology, 55: 497.

Gomez, K.A., A.A. Gomez, 1984. Statistical Procedures for Agricultural Research. New York, USA: John Wiley.

Hartman, K.M., J.N. Sasser, 1985. Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology. – In: Barker, K. R., Carter, C. C. and Sasser, J.N. (Eds.). An advanced treatise on *Meloidogyne,* Vol. II, Methodology. Raleigh, NC, USA, North Carolina State University Graphics, 69-77.

Hussey, R.S., K.R. Baker, 1973. A comparison of methods of collecting inocula of *Meloidogyne* species, including a new technique. – Plant Disease Report, 57:1025-1028.

Hussey, R.S., V.M. Williamson, 1997. Physiological and molecular aspects of nematode parasitism. – In: Barker KR, Pederson GA, Windham GL, eds. Plant and Nematode Interactions.

Jatala, P., J. Bridge, 1990. Nematode parasites of root and tuber crops. – In: Luc M, Sikora, R.A., J. Bridge, eds. Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. Wallingford, UK: CAB International, 137–180.

MacGuidwin, A.E., D.I. Rouse, 1990. Effect of *Meloidogyne hapla*, alone and in combination with subthreshold population of *Verticillium dahliae*, on disease symptomology and yield of potato. – Phytopathology, 80: 482-486.

Olthof, T.H.A., J.W. Potter, 1972. Relationship between Population Densities of Meloidogyne hapla and Crop Losses in Summer-Maturing Vegetables in Ontario. – Phytopathology, 62: 981-986.

Samaliev, H., O. Baycheva, 2010. Distribution of Root-Knot Nematodes (Genus *Meloidogyne* Goeldi) on the Potatoes in the Plovdiv, Troyan and Samokov Regions in Bulgaria. Theoretical and practical problems of parasitology. Russian Academy of Science, 458-465.

Samaliev, H., 1997. Incidence of the root knot nematode Meloidogyne hapla Chitwood in potato in Bulgaria. – In: Higher School of Agriculture - Plovdiv, Scientific Works, vol. XLII, book 3, 121-124.

Samaliev, H., 2009. Races of Four Species of Root-knot Nematodes of Meloidogyne Goeldi on Vegetable Crops in Glasshouses in Bulgaria. – Plant Science, 46(6), 542-547.

Samaliev, H., 2011. Plant-parasitic nematodes associated with potatoes (Solanum tuberosum L.) in Bulgaria. – Plant Science, 48 (5), 510-514.

Samaliev, H., D. Stoyanov, 2007. Parasitic Nematodes of Crop Plants and Their Control. Agricultural academic press, Plovdiv, p. 328.

Santo, G.S., J.H. O'Bannon, A.P. Nycepir, R.P. Ponti, 1981. Ecology and control of root-knot nematodes on potato. – In: Proceedings of the 20th Annual Washington Potato Conference, 3–5 February, Moses Lake,Washington, USA. Moses Lake, WA, USA: Washington State Potato Commission, 135–139.

Scurrah, M., B. Niere, J. Bridge, 2005. Nematode Parasites of Solanum and Sweet potatoes. – In: Luc M, Sikora, R.A., J. Bridge, eds. Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. Wallingford, UK: CAB International, 193-221.

Stoyanov, D., 1980. Plant parasitic nematodes and their control, Zemizdat, S., 220.

Taylor, A.L., J.N. Sasser, 1978. Biology, identification and control of root knot nematode (*Meloidogyne* spp.). North California State University graphs, Releigh, N.C., p. 111.

Van der Beek, J.G., P.F.G. Vereijken, L.M. Poleij, C.H. Van Silfhout, 1997. Isolate-by-cultivar interaction in root-knot nematodes *Meloidogyne hapla*, *M. chitwoodi*, and *M. fallax* on potato. – Canadian Journal of Botany, 76, 75–82.

Vovlas, N., G. Grammatikaki, A. Sonnino, 1994. Response of anther culture-derived diploid lines of potato to the root-knot nematode *Meloidogyne incognita*. – Nematologia Mediterranea, 22, 237–240.

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